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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

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Applicant's or agent's file reference SJB:SDT:KR:FP13129	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. **PCT/AU00/00864**	International Filing Date *(day/month/year)* 19 July 2000	Priority Date *(day/month/year)* 19 July 1999

International Patent Classification (IPC) or national classification and IPC

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Applicant

ST. VINCENT'S INSTITUTE OF MEDICAL RESEARCH et al

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

[X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of **83** sheet(s).

3. This report contains indications relating to the following items:

I	[X]	Basis of the report
II	[ ]	Priority
III	[ ]	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	[ ]	Lack of unity of invention
V	[X]	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	[ ]	Certain documents cited
VII	[ ]	Certain defects in the international application
VIII	[X]	Certain observations on the international application

Date of submission of the demand	Date of completion of the report
17 October 2000	6 November 2001
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **J.H. CHAN** Telephone No. (02) 6283 2340

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU00/00864

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed.

☒ the description, pages **1-3, 6-16, 22, 25-39, 42, 45-57,** as originally filed,
pages **4A,** received on **28 February 2001** with the letter of **28 February 2001**
pages **5, 17-21, 23, 24, 40, 41, 43 and 44** received on **24 September 2001** with the letter of **24 September 2001,**
pages **4,** received on **30 October 2001** with the letter of **30 October 2001.**

☒ the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages **58-62,** received on **24 September 2001** with the letter of **24 September 2001.**

☒ the drawings, pages **1-37,** as originally filed,
pages , filed with the demand,
pages , received on with the letter of

☒ the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages **1-64,** received on **30 October 2001** with the letter of **30 October 2001.**

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☒ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☒ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages

☐ the claims, Nos.

☐ the drawings, sheets/fig.

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims **1-39**	**YES**
	Claims	**NO**
Inventive step (IS)	Claims **1-39**	**YES**
	Claims	**NO**
Industrial applicability (IA)	Claims **1-39**	**YES**
	Claims	**NO**

2. Citations and explanations (Rule 70.7)

The follwing documents have been considered in the preparation of this international preliminary examination report:

D1 Genbank accession no. AF121352.
D2 Genbank accession no. AF192526.
D3 Genbank accession no. AF133299.
D4 Genpept accession no. AAD22055.
D5 WO 9828423 A.
D6 AU 702557 (46773/96) B.
D7 AU 718458 (38661/97) B.
D8 WO 97/23614 A.
D9 Endocrine Review 20 no. 3 pages 345-357 (1999).
D10 Life Sciences volume 65 no 11 pages 1087-1102 (1999).

New citation:

D11 Genpept accession no. AAD22055 (published 15 June 1999)

Novelty and inventive step:

None of the documents D1-D11 discloses lectin-like proteins and the nucleotide molecules with the sequences and antibodies to the proteins as defined in claims of this international application. As such the invention as defined in claims 1-39 is novel and inventive in the light of these documents.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

The lengths of the sequences seq id nos 45 and 46 described on page 38 (lines 4-6) appear to be inconsistent with those provided in the sequence listings (page 57-59 of the sequence listings). The former passage defines seq id nos 45 as being 937 bp and seq id no 46 being 845 bp in length; however the latter defines them as 845 and 937 bp respectively.

It appears that when the molecule is expressed on the osteoblast cell membrane it is not secreted. Preventing expression of the factor results in increased formation of mononuclear precursors of osteoclasts.

SUMMARY OF THE INVENTION

In a first aspect, the invention provides a nucleic acid molecule which comprises a sequence encoding a protein which

a) is expressed at least on osteoblasts, and inhibits osteoclast differentiation from haematopoietic cell precursors,

or which hybridises to said nucleic acid molecule under stringent conditions.

Suitable stringent conditions are well known in the art. See the well known textbook by Sambrook *et al* (1989), and Example 2 herein.

The nucleic acid may be cDNA, genomic DNA or messenger RNA. Preferably the nucleic acid molecule is a cDNA. More preferably the cDNA comprises a sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11, SEQ ID NO: 33, SEQ ID NO: 36, SEQ ID NO: 44, SEQ ID NO: 45 and SEQ ID NO: 46.

Preferably the protein inhibits differentiation of haematopoietic cells to osteoclast cells. In a particularly preferred embodiment, the nucleic acid molecule of the invention comprises a 110 base pair sequence as set out in SEQ ID NO: 2.

This aspect of the invention also encompasses anti-sense sequences directed against the nucleic acid molecule defined above, and particularly encompasses an anti-sense sequence directed against SEQ ID NO: 10. Preferably the anti-sense sequence is SEQ ID NO: 24 or SEQ ID NO: 25.

In a second aspect, the invention provides a polypeptide encoded by the nucleic acid molecule of the

invention. Preferably the polypeptide is encoded by the human cDNA sequence. More preferably the polypeptide comprises an amino acid sequence encoded by SEQ ID NO: 20.

In a third aspect, the invention provides an antibody directed against a polypeptide of the invention. Preferably the antibody is directed against an epitope present in a sequence selected from the group consisting of

H-Cys-Met-Ala-Gln-Glu-Ala-Gln-Leu-Ala-Arg-Phe-Asp-Asn-Gln-Asp-Glu-Leu-Asn-Phe-OH (SEQ ID NO: 26),

H-Cys-Val-Thr-Lys-Ala-Ser-Leu-Pro-Met-Leu-Ser-Pro-Thr- Gly-Ser-Pro-Gln-Glu-NH_2 (SEQ ID NO: 48), and

H-Cys-Val-Gln-Lys-Pro-Glu-Glu-Gly-asn-Gly-Pro-Leu-Gly-Thr-Gly-Asp-NH_2 (SEQ ID NO: 49).

The antibody may be polyclonal or monoclonal, but is preferably monoclonal. Suitable methods for generating either polyclonal or monoclonal antibodies are very well known in the art. It will be clearly understood that the invention encompasses biologically-active fragments and analogues of such antibodies, including but not limited to ScFv fragments, trimeric antibodies, humanised antibodies and the like. Again, methods for producing such active fragments and analogues are well known in the art. See for example PCT/AU93/00491 and PCT/AU97/00212 and references cited therein.

In a fourth aspect, the invention provides a composition comprising a polypeptide or an antibody of the invention, together with a pharmaceutically-acceptable carrier.

Methods and pharmaceutical carriers for preparation of pharmaceutical compositions are well known in the art, for example as set out in textbooks such as Remington's Pharmaceutical Sciences, 17th Edition, Mack Publishing Company, Easton, Pennsylvania, USA.

sequences of exons I and II were completely different to that of mOCIL exon 1. The sequence of exons III to VI was 90.28% identical to that of mOCIL from exon II. The 5' flanking region adjacent to exon I is a GC-rich region, containing a Sp 1 binding site. In combination, these features indicated that in fact this was a different gene. A search of the GenBank database showed that exons I and II showed 100% identity to a cDNA clone encoding a C-type lectin expressed in mouse bone marrow-derived dendritic cells, which was deposited in the GenBank data base on 20 January 1999 (Accession No. AF121352) and released on 15 June 1999. However, exons III to VI are 92% identical to AF121352 cDNA. The sequence of this genomic clone is redesignated as mOCIL-related protein 1 (mOCILrP1) gene (SEQ ID NO:11). The full length mOCILrP1 cDNA was originally thought to be a spliced variant of mOCIL, and is 990 bp in length (SEQ ID NO: 12).

To confirm the mOCIL (SEQ ID NO: 36) and mOCILrP1 (SEQ ID NO:12) cDNA sequences, RT-PCR was carried out using total RNA isolated from ST2 mouse stromal cells, primary mouse calvarial osteoblasts and mouse liver tissue. The sense primer represented nucleotides 18-36 of mOCILrP1 (SEQ ID NO:12), and is designated as primer OCILm47 (SEQ ID NO: 13),

OCILm47 5'- TCC CAT GCC AGA TTG CTT G-3'

The antisense primer, which was originally designed from mOCIL2kb (SEQ ID NO: 10) nucleotides 136-157, represented nucleotides 746-725 of mOCIL (SEQ ID NO: 36) and is designated primer OCILm12 (SEQ ID NO: 14),

OCILm12 5'-GGG ACC ATA GGG GAA AGA GTA G-3'

The PCR was run at 94°C for 5 min, then 35 cycles of 94°C for 30s, 60°C for 30s, and 72°C for 1 min, followed by a final extension step of 72°C for 10 min. Seven clones

containing a 721 bp fragment were obtained from three sources, ST2 mouse stromal cells, primary mouse calvarial osteoblasts and mouse liver tissue. In 2 of the 7 clones, there was 100% identity to mOCILrP1 sequence, and 92.2% identity to mOCIL after the first 115 bp. In the other 5 clones, when compared to the mOCILrP1 sequence, there was 100% identity in the first 106 bp (exons I and II), but only 90.5% identity in the remaining 615 bp. This 721 bp fragment, originally designated as mOCIL47, was redesignated as mOCILrP2 (SEQ ID NO: 15). MOCILrP2 is related to, but distinct from, mOCIL (SEQ ID NO: 36) and mOCILrP1 (SEQ ID NO: 12).

A sense primer representing nucleotides 343-364 of mOCIL2kb (SEQ ID NO:10) and representing nucleotides 34-57 of mOCIL (SEQ ID NO:36), designated as OCILm17 (SEQ ID NO: 16),

OCILm17 5'-TGG AAA CTC AGC TCC TCA GCT CTG-3'

and antisense primer OCILm12 were also used to carry out RT-PCR with RNA from three sources, ST2 mouse stromal cells, primary mouse calvarial osteoblasts and mouse liver tissue, as above. PCR was run under the same conditions as above. Ten clones were obtained, each containing a 713 bp fragment. This sequence is designated mOCIL17 (SEQ ID NO: 17), and is 100% identical to mOCIL (SEQ ID NO: 36).

RT-PCR was also carried out using a sense primer corresponding to the region located at the junction of exons II and III, representing nucleotides 245-269 of mOCIL (SEQ ID NO: 36) and at the junction of exon III and exon IV, representing nucleotides 243-267 of mOCILrP1 (SEQ ID NO: 12), and designated primer OCILm32 (SEQ ID NO: 18),

OCILm32 5'- TTT GTC AGC AAC AAA GAC AGA ACA G-3'

The primer oligonucleotide OCILm32 has 23 of 24 bp complementary to mOCILrP1:

5'-TTTGTCAGCAACAAAGACAGAACAG-3' Primer
||||||| ||||||||||||||||
3'-AAACAGTCATTGTTTCTGTCTTGTC-5' mOCILrP1 (267) Strand -

Primer OCILm12 was used as an antisense primer. RT-PCR was carried out with RNA from three sources, ST2 mouse stromal cells, primary mouse calvarial osteoblasts and mouse liver tissue, as above. PCR was run under the same conditions. Four clones were obtained, each containing a 502 bp fragment. Three of the four clones have 100% identity to mOCIL (SEQ ID NO: 36) and one of the four clones is 100% identical to mOCILrP1 (SEQ ID NO: 12).

RT-PCR was also carried out using the sense primer OCILm47 (SEQ ID NO: 13) and an antisense primer representing nucleotides 855-874 of mOCILrP1 (SEQ ID NO: 12), designated primer OCILm49 (SEQ ID NO: 38),

OCILm49 5'-GTG GTT GCT CAG ATG TGA AC-3'

RT-PCR was carried out with RNA from the same three sources, ST2 mouse stromal cells, primary mouse calvarial osteoblasts and mouse liver tissue, as above. PCR was run under the same conditions. Two clones were obtained, each containing a 856 bp fragment with 100% identity to AF121352 and in which the first 713 bp are 100% identical to mOCILrP2 (SEQ ID NO: 15).

To further confirm that mOCILrp2 is AF121352, an antisense primer was designed based on the sequence of AF121352 (nucleotides 908-929), designated as primer OCILm48 (SEQ ID NO: 39),

OCILm48 5'-TTC ACA CAT CCC AGA AGA GGA C-3'

OCILm47 (SEQ ID NO: 13) was used as sense primer. RT-PCR was carried out under same conditions as above. Two clones were obtained, each containing a 916 bp fragment which has

100% identity to AF121352 and in which the first 713 bp is 100% identical to mOCILrP2 (SEQ ID NO: 15).

The full length mOCILrP2 cDNA is 988 bp in length. Its first 123 bp is 100% identical to mOCILrP1, but only 91.7% identical in the remaining 865 bp. Figure 7 summarises the homology between mOCIL, mOCILrP1 and mOCILrP2. The three different sequences (SEQ ID NO: 12, 15 and 36), which overall have 87% identity, may represent gene duplications, where either one or all three sequences may have similar biological outcomes. The functional data we have to date, relating to the inhibition of osteoclast formation from haemopoietic precursor cells using antisense oligonucleotides (SEQ ID NO: 24 and 25), have been obtained mainly with mOCIL17 (SEQ ID NO: 17), although experiments with recombinant protein (see below) indicate that the extracellular domains of mOCIL, mOCILrP1 and mOCILrP2 respectively can inhibit osteoclast formation.

mOCIL has an open reading frame encoding a 207 amino acid protein. As shown in Figure 8a, its putative protein structure is typical of a type II membrane protein, with a predicted 143 amino acid extracellular domain, a 21 amino acid transmembrane domain, and a 43 amino acid cytoplasmic domain. The extracellular domain has 5 cysteine residues. There are three potential N-linked glycosylation sites at residues 74, 100 and 158, all of which are in the extracellular domain. The putative protein sequence for mOCIL is designated mOCIL protein (SEQ ID NO: 40).

Comparison of the putative protein sequences derived from the rOCIL323, rOCIL1.3kb and mOCIL cDNA sequences with the SwissProt protein database indicated that the mOCIL protein sequence included an 113 amino acid C-lectin type motif, from positions 80 to 192 in the mOCIL protein sequence (SEQ ID NO: 40). This C-lectin motif is similar to that of CD69, a membrane-bound lectin expressed by bone marrow haematopoietic cells, and thought to be involved in monocyte differentiation. C-lectin motifs are

also involved in cell-cell contact and lipid binding (Sharon and Lis, 1995; Gabius 1997; Kieda, 1998).

mOCILrP1 has an open reading frame encoding a 218 amino acid protein. The putative protein sequence for mOCILrP1 is designated mOCILrP1 protein (SEQ ID NO: 41). Its structure is also typical of a type II membrane protein, with a predicted 142 amino acid extracellular domain, a 21 amino acid transmembrane domain, and a 55 amino acid cytoplasmic domain. The mOCILrP1 protein sequence also has a 113 amino acid C-lectin type motif, from positions 92 to 204 in the mOCILrP1 protein sequence (Figure 8b). The extracellular domain has 6 cysteine residues. There are three potential N-linked glycosylation sites at residues 86, 112 and 207, all of which are in the extracellular domain. There is a myristylation motif in the intracellular domain.

MOCILrP2 has an open reading frame encoding a 217 amino acid protein. The putative protein sequence for mOCILrP2 is designated mOCILrP2 protein (SEQ ID NO: 42). Its structure is also that of a type II membrane protein, with a predicted 141 amino acid extracellular domain, a 21 amino acid transmembrane domain, and a 55 amino acid cytoplasmic domain. Similarly to mOCIL and mOCILrP1, the mOCILrP2 protein sequence has an 113 amino acid C-lectin type motif, from positions 92 to 204 in the mOCILrP2 protein sequence (Figure 8c). The extracellular domain has 6 cysteine residues. There are four potential N-linked glycosylation sites at residues 86, 95, 112 and 165, all of which are in the extracellular domain.

The three different mouse protein sequences (SEQ ID NO: 40, 41 and 42) overall have 89% identity as shown in Figure 9. There are differences in the intracelullar domains between mOCIL and mOCIL-related proteins, and these domains may have different functional roles. If the C-type lectins act as receptors, the intracellular domains may confer different properties as a result of signal transduction. Comparison of the protein sequences in the

for 15min. Eight positive clones were obtained after tertiary screening. Clone No. 6 is a 1.3 kb cDNA segment, whose sequence was designated hOCIL clone 6 (SEQ ID NO: 19). The putative protein sequence encoded by bp883-1059 was a C-type lectin moiety, which showed 73% homology to the C-type lectin sequence previously demonstrated in rOCIL323, rOCIL1.3kb and mOCIL2kb. However, regions of amino acid sequence 5' and 3' to this C-type lectin domain were different from those of the mouse and rat sequences, as shown in Figure 6.

Clone No. 8 is 960 bp long. It has 64% identity over a length of 145 bp with rOCIL402. A search of the EST database showed that clone No. 8 has 99.5% sequence identity with an EST clone of unknown function from human pregnant uterus, Accession No. AA029932, over the published length of this EST 209 bp. This EST clone was ordered and further sequenced. The EST clone is 680 bp in length, and has 64% identity with rOCIL1.3kb over a length of 343 bp. It also has 64% identity over a length of 346 bp compared to mOCIL. RT-PCR showed that clone No 8 and AA029932 represent overlapping clones, which are contiguous, and combine to represent a human OCIL clone 1 of 1305 bp in length (SEQ ID NO: 20).

The deduced protein sequence has 56% homology to the deduced protein sequence of rOCIL1.3kb, and 62% homology to that of mOCIL. These differences are principally at the N-terminus. Although there is 80% homology between the mouse and human OCIL proteins in certain regions, this indicates that the mouse cDNA could not reliably be used to isolate a human genomic DNA encoding hOCIL.

In order to obtain the hOCIL gene, the 680 bp cDNA insert of clone AA029932 was isolated and screened by Genome Systems, Inc. against the genomic BAC Human Release II Hybridisation library, as described in Example 2. One positive clone was obtained. This genomic sequence, corresponding to the sequence from 654bp-1304bp of hOCIL,

has 100% identity to a sequence segment within a human genomic clone OF 178,607 bp, which was deposited in the GenBank database on April 6, 1999 (Accession No. AC007068), and the 5' flanking region, promoter region and the first 654 bp of cDNA sequences are represented in the 74,801 bp sequence deposited in the GenBank database on December 9, 1999 (Accession No. AC010186).

The hOCIL gene is located in chromosome 12p. Chromosome 12 and chromosome 11 are considered to be evolutionarily related. There are several examples of evolutionarily related proteins whose genes are located on chromosome 12 and chromosome 11, such as PTH and PTHrP, IGF and IGF I , Harvey ras sarcoma 1, and Kisten ras sarcoma 2, etc. (Martin et al., 1991). Thus chromosome 11 and chromosome 12 share genes of similar biological characteristics with redundant function.

Example 4: *Effect of Anti-Sense Oligonucleotides on Osteoclast Formation*

Primary mouse calvarial osteoblasts were cocultured with mouse bone marrow cells to generate mononuclear and multinucleate osteoclasts. Staining for tartrate-resistant acid phosphatase (TRAP), performed using a commercial leukocyte acid phosphatase kit from Sigma Diagnostics (St. Louis, MO, USA; Katsogiannis *et al*, 1998), identified these cells as osteoclasts. Under normal conditions, multinucleate functional osteoclasts are not formed unless the cocultures are stimulated with 1,25-dihydroxyvitamin D_3 and PGE_2.

Experiments were carried out to block translation of OCIL mRNA in order to determine the function of its translated product. Antisense oligonucleotides may also down-regulate mRNA levels, and thus may effectively decrease transcription as well as translation. Primary mouse calvarial osteoblasts were treated with antisense oligonucleotides. Four antisense oligonucleotide sequences were designed. Two of these antisense oligonucleotide

Example 9: *Antibodies Directed Against OCIL*

The following peptide fragment of the deduced amino acid sequence derived from the cDNA sequence of mOCIL17 (SEQ ID NO: 17) was synthesized, and was used to immunize rabbits, using standard protocols.

H-Cys-Met-Ala-Gln-Glu-Ala-Gln-Leu-Ala-Arg-Phe-Asp-Asn-Gln-Asp-Glu-Leu-Asn-Phe-OH (SEQ ID NO: 26)

This peptide sequence showed a high homology to mOCILrP1 (SEQ ID NO: 41) and mOCILrP2 (SEQ ID NO: 42), as follows:

```
1      CMAQEAQLARFDNQDELN
       ||||||||||||||||||
108    CMAQEAQLARFDNQDELN   mOCIL (SEQ ID NO: 40)

1      CMAQEAQLARFDNQDELN
       ||||||||||||  |||
120    CMAQEAQLARFDNEKELN   mOCILrP1 (SEQ ID NO: 41)

1      CMAQEAQLARFDNQDELN
       ||||||||||||  ||
120    CMAQEAQLARFDNEEELI   mOCILrP2 (SEQ ID NO: 42)
```

Two specific peptide fragments of the deduced amino acid sequence derived from the cDNA sequence of mOCIL (SEQ ID NO: 36) and mOCILrP1/mOCILrP2 (SEQ ID NO: 12 and 15) in the intracellular domain were synthesised, and were also used to raise antibodies:

Antibody MOCIL-3 is specific for an epitope in the following sequence of mOCIL:

H-Cys-Val-Thr-Lys-Ala-Ser-Leu-Pro-Met-Leu-Ser-Pro-Thr-Gly-Ser-Pro-Gln-Glu-NH_2 (SEQ ID NO: 48)

Antibody MOCIL-RP-1 is specific for an epitope in the following sequence of mOCILrP1/mOCILrP2:

H-Cys-Val-Gln-Lys-Pro-Glu-Glu-Gly-asn-Gly-Pro-Leu-Gly-Thr-Gly-Asp-NH_2 (SEQ ID NO: 49)

The antibodies raised may be used to detect the OCIL protein, using standard immunohistochemical methods, or to neutralize OCIL activity in murine co-cultures to stimulate osteoclast formation.

Example 10: Immunohistochemistry

Rabbit polyclonal antibodies prepared as described in Example 7 were used for immunohistochemistry. A kit for the standard peroxidase-labelled streptavidin-biotin detection method (DAKO, Boenisch, 1989) was used according to the manufacturer's instructions, with minor modifications. The dilution of the antiserum used was optimised in preliminary experiments. Incubation of tissue sections with a 1:100 dilution of the primary antiserum was carried out overnight at 4°C in a humidified chamber. Peroxidase activity was detected with 3'-3'-diamino-benzidine tetrahydrochloride (Sigma) and 0.15% H_2O_2. Slides were counterstained with haematoxylin, dehydrated and mounted on a coverslip. The tissue expression of mOCIL, mOCILrP1 and mOCILrP2 proteins as detected using the 3 antibodies raised against the sequences SEQ ID NO: 26; SEQ ID NO: 48 and SEQ ID NO: 49 was identical. The results are summarized in Table 4.

Example 11: Production of Recombinant OCIL protein in a mammalian expression system

OCIL proteins were prepared by recombinant DNA technology to allow more extensive laboratory studies of their actions on osteoclast formation as well as osteoblast function. Soluble mouse and rat OCIL cDNA tagged at the N-terminus with the FLAG epitope were constructed in the pEF-BOS Mammalian expression vector (Mizushima & Nagata 1990), which had been modified to contain an in-frame IL-3 signal sequence and FLAG peptide coding sequence (gift of Dr. D Hilton).

In order to obtain a RT-PCR product encoding the mOCIL (SEQ ID NO: 36) extracellular domain (amino acids 63-207) to clone into the MluI site of the vector, as shown in Figure 19a, the RT-PCR was carried out using total RNA isolated from primary mouse calvarial osteoblasts, which support osteoclast differentiation in coculture. A sense primer, OCILm33, comprising OCILm32 representing nucleotides 245-269 of mOCIL (SEQ ID NO: 36) and containing a MluI site, designated primer OCILm33 (SEQ ID NO: 27):

OCILm33 5'-GCC ACG CGT TTG TCA GCA ACA AAG ACA GAA CAG-3'

and an antisense primer representing nucleotides 746-725 of mOCIL (SEQ ID NO: 36) and containing a MluI site, designated primer OCILm46 (SEQ ID NO: 28),

OCILm46 5'-GCC ACG CGT GGG ACC ATA GGG GAA AAA GTA G-3'

were used as primers in the PCR. PCR was run at 94°C for 5 min, then 35 cycles of 94°C for 30 s, 60°C for 30s, and 72°C for 1 min, followed by a final extension step of 72°C for 10 min. A 501 bp fragment was obtained and further cloned into the expression vector pEF-BOS. The open reading frame and FLAG fusion was confirmed by sequencing (bp 1-132), and the 501 bp fragment sequence (SEQ ID NO: 29) was confirmed to be identical to mOCIL17 (SEQ ID NO: 17). The

sequencing results also showed that the primer OCILm46 had 22 of 23 bp complementary to mOCIL17 (SEQ ID NO: 17) and mOCIL (SEQ ID NO: 36):

5'-GCCACGCGTGGGACCATAGGGGAAAAAGTAG-3' Primer OCILm46

3'-ATCGTGAAACCCTGGTATCCCCTTTCTCATC-5' mOCIL (725) Strand +

To obtain a PCR product encoding the rOCIL1.3kb (SEQ ID NO: 7) extracellular domain (amino acids 40-179), a sense primer to represent nucleotides 126-146 of rOCIL1.3kb with the MluI site, designated primer OCILr22 (SEQ ID NO: 30),

OCILr22 5'- GCC ACG CGT TCA GTA AAA AAG ACA GCC AAG-3'

and an antisense primer representing nucleotides 544-526 of rOCIL1.3kb with the MluI site, and designated primer OCILr23 (SEQ ID NO: 31),

OCILr23 5'-GCC CAG CGT AAC TAC AGG CAC TGT GAG G-3'

were used as primers in a PCR. PCR was carried using rOCIL1.3 kb plasmid as a template and run at 94°C for 5 min, then 35 cycles of 94°C for 30 s, 60°C for 30s, and 72°C for 1 min, followed by a final extension step of 72°C for 10 min. A 421 bp fragment was obtained and cloned into the expression vector pEF-BOS, as shown in Figure 19b. The open reading frame and FLAG fusion were confirmed by sequencing.

HEK 293 cells were transfected with both mouse and rat expression constructs using Lipofectamine (Life Technologies, Inc). Supernatant was harvested after 72 hours. The recombinant protein was purified by incubation with the anti-FLAG M2 affinity gel (Kodak), and eluted with the FLAG peptide (Kodak) as outlined in the manufacturer's protocol. The purified protein was used to

CLAIMS

1. An isolated nucleic acid molecule which comprises a sequence encoding a type II membrane polypeptide expressed on the osteoblast cell surface, selected from the group consisting of osteoclast inhibitory lectin (OCIL) and OCIL-related protein, which

a) is expressed at least on osteoblasts, and

b) inhibits osteoclast differentiation from haematopoietic cell precursors.

2. A nucleic acid molecule according to claim 1, which is a cDNA.

3. A nucleic acid molecule according to claim 1 or claim 2, which is of human, mouse or rat origin.

4. A nucleic acid molecule according to claim 2, in which the cDNA comprises a sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 15, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 33, SEQ ID NO: 36, SEQ ID NO: 44, SEQ ID NO: 45 and SEQ ID NO: 46.

5. A nucleic acid molecule according to claim 1, which is a gDNA.

6. A nucleic acid molecule according to claim 5, in which the gDNA comprises a sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 21, and SEQ ID NO: 37, or which hybridises to said nucleic acid molecule under stringent conditions.

7. A nucleic acid molecule according to any one of claims 1 to 6, which encodes an extracellular domain of an OCIL or of an OCIL-related protein.

8. A nucleic acid molecule according to any one of claims 1 to 7, which encodes a protein which inhibits differentiation of haematopoietic stem cells to osteoclast progenitor cells.

9. A nucleic acid molecule according to any one of claims 1 to 8, which comprises a 110 base pair sequence as set out in SEQ ID NO: 2.

10. An anti-sense sequence directed against a nucleic acid molecule according to any one of claims 1 to 9.

11. An anti-sense sequence according to claim 10, directed against SEQ ID NO: 10.

12. An anti-sense sequence according to claim 10 or claim 11, which is SEQ ID NO: 24 or SEQ ID NO: 25.

13. An isolated polypeptide encoded by a nucleic acid molecule according to any one of claims 1 to 9.

14. A polypeptide according to claim 13, which is encoded by the human cDNA or gDNA sequence.

15. A polypeptide according to claim 13, which is encoded by the mouse cDNA or gDNA sequence.

16. A polypeptide according to claim 15, comprising a sequence selected from the group consisting of SEQ ID NO: 40, SEQ ID NO: 41, and SEQ ID NO: 42.

17. A polypeptide according to claim 13, which comprises an amino acid sequence encoded by SEQ ID NO: 20.

18. An isolated polypeptide selected from the group consisting of a C-lectin motif, an extracellular domain, a transmembrane domain, or a cytoplasmic domain of a polypeptide according to any one of claims 13 to 17.

19. An antibody directed against a polypeptide according to any one of claims 13 to 18.

20. An antibody according to claim 19, which is directed against an epitope present in a sequence selected from the group consisting of
H-Cys-Met-Ala-Gln-Glu-Ala-Gln-Leu-Ala-Arg-Phe-Asp-Asn-Gln-Asp-Glu-Leu-Asn-Phe-OH (SEQ ID NO: 26).
H-Cys-Val-Thr-Lys-Ala-Ser-Leu-Pro-Met-Leu-Ser-Pro-Thr- Gly-Ser-Pro-Gln-Glu-NH_2 (SEQ ID NO: 48), and
H-Cys-Val-Gln-Lys-Pro-Glu-Glu-Gly-asn-Gly-Pro-Leu-Gly-Thr-Gly-Asp-NH_2 (SEQ ID NO: 49).

21. An antibody according to claim 19 or claim 20, which is monoclonal.

22. A composition comprising a polypeptide according to any one of claims 13 to 18, together with a pharmaceutically-acceptable carrier.

23. A composition comprising an antibody according to any one of claims 19 to 21, together with a pharmaceutically-acceptable carrier.

24. A method of treatment of a condition characterised by abnormal bone resorption, comprising the step of administering an effective amount of a modulator of expression or function of a polypeptide according to any one of claims 13 to 18.

25. A method according to claim 24, in which the condition involves excessive bone resorption, and the method comprises administration of a polypeptide according to any one of claims 13 to 18, or a nucleic acid encoding this polypeptide, or encoding a biologically-active fragment or analogue thereof.

26. A method according to claim 25, in which the condition is selected from the group consisting of osteoporosis, primary hyperparathyroidism, Paget's disease, rheumatoid arthritis, renal osteodystrophy, humoral hypercalcaemia of malignancy, and conditions where cancer has metastasised to bone.

27. A method according to claim 24, in which the condition involves deficient bone resorption, and the method comprises administration of an antibody according to any one of claims 19 to 21 or an anti-sense oligonucleotide according to any one of claims 10 to 12.

28. A method according to claim 26, in which the condition is osteopetrosis.

29. A method of promoting healing of bone fractures, particularly in an individual in whom fracture healing is delayed or deficient, comprising the step of administering an effective amount of a polypeptide according to any one of claims 13 to 18.

30. A method according to claim 29, in which the individual is suffering from osteoporosis or diabetes mellitus.

31. A method of modulating breast and/or lymph node development, comprising the step of administering an

effective amount of a modulator of expression or function of a polypeptide according to any one of claims 13 to 18 to a subject in need of such treatment.

32. A diagnostic kit for detection of abnormalities in the structure, expression or control of a type II membrane polypeptide expressed on the osteoblast cell surface, selected from the group consisting of osteoclast inhibitory lectin (OCIL) and OCIL-related protein, comprising a reagent selected from the group consisting of

(a) a nucleic acid according to any one of claims 1 to 9, or a fragment thereof capable of hybridising to a nucleic acid according to any one of claims 1 to 9;

(b) an anti-sense nucleic acid according to any one of claims 10 to 12;

(c) a polypeptide according to any one of claims 13 to 18, and

(d) an antibody according to any one of claims 19 to 21.

33. A diagnostic kit according to claim 32, in which the reagent is labelled with a detectable marker.

34. A method of screening of candidate agents for treatment of a condition characterised by abnormal bone resorption, comprising the step of assessing the ability of each agent to modulate expression or function of a polypeptide according to any one of claims 13 to 18.

35. An oligonucleotide primer selected from the group consisting of antisense primers having the sequence set out in SEQ.ID. NO:5, 6, 30, 35, 13, 16, 18, 27, 47, 50, 52, 54, or 55, and sense primers having the sequence set out in SEQ.ID. NO: 3, 31, 32, 14, 28, 34, 38, 39,51, 53, 22, 23, 24, 25, 43 or 56.